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P L S S I F S R I G D P
CCC ATA TCG TCA ATC TTC TCG AGG ATT GGG GAC CCT
T

Fig. 1

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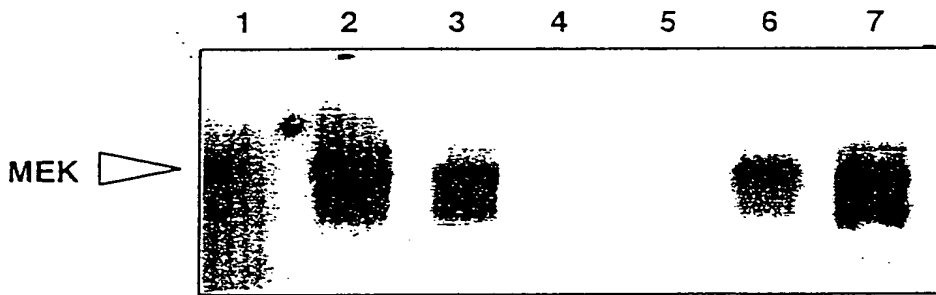


Fig. 2

Conservation of PreS2-TLM between various HBV subtypes

Nucleotide sequences, amino acid sequences and hydropathy values of the amino acid side chains (according to Kyte & Doolittle, 1982) of PreS2-TLM from subtype ayw (1) as compared to six other HBV subtypes ayw (2), adr (1), adr (2), ayr, adw and adw2. The amino acids identical with the sequence of PreS2-TLM of subtype-ayw (1) and the associated hydropathy values are shown in boldface.

Subtype ayw (1)

| | | | | | | | | | | | |
|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| CCC | TTA | TCG | TCA | ATC | TTC | TCG | AGG | ATT | GGG | GAC | CCT |
| Pro | Leu | Ser | Ser | Ile | Phe | Ser | Arg | Ile | Gly | Asp | Pro |
| -1.6 | 3.8 | -0.8 | -0.8 | 4.5 | 2.8 | -0.8 | -4.5 | 4.5 | -0.4 | -3.5 | -1.6 |

Subtype ayw (2)

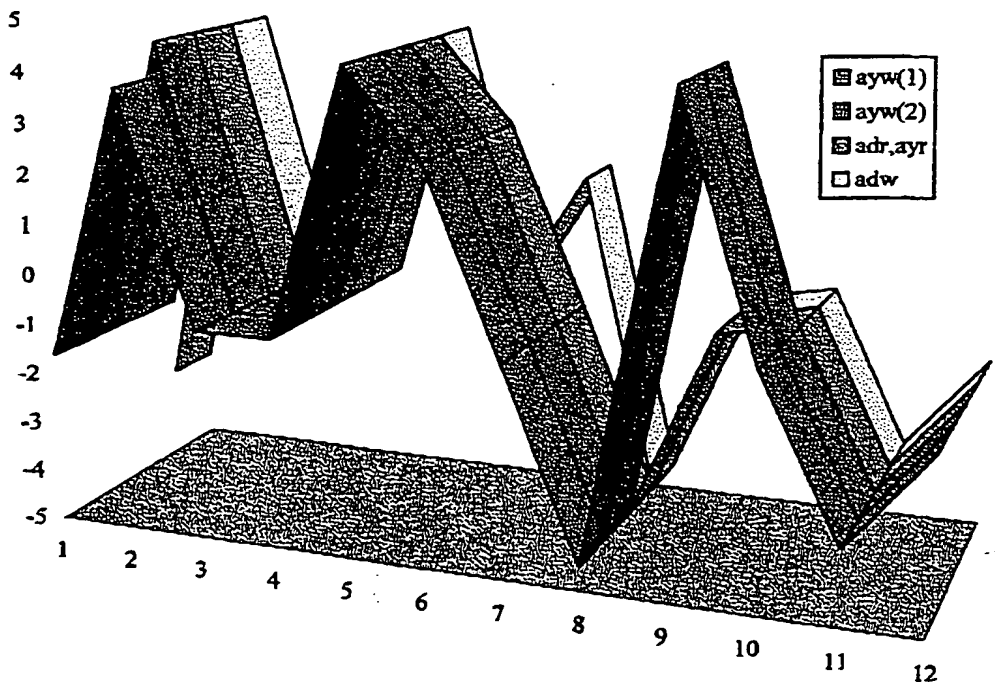
| | | | | | | | | | | | |
|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| CCC | ATA | TCG | TCA | ATC | TTC | TCG | AGG | ATT | GGG | GAC | CCT |
| Pro | Ile | Ser | Ser | Ile | Phe | Ser | Arg | Ile | Gly | Asp | Pro |
| -1.6 | 4.5 | -0.8 | -0.8 | 4.5 | 2.8 | -0.8 | -4.5 | 4.5 | -0.4 | -3.5 | -1.6 |

Subtypes adr (1), adr (2), ayr

| | | | | | | | | | | | |
|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| CCC | ATA | TCG | TCA | ATC | TTC | TCG | AGG | ACT | GGG | GAC | CCT |
| Pro | Ile | Ser | Ser | Ile | Phe | Ser | Arg | Thr | Gly | Asp | Pro |
| -1.6 | 4.5 | -0.8 | -0.8 | 4.5 | 2.8 | -0.8 | -4.5 | -0.7 | -0.4 | -3.5 | -1.6 |

Subtypes adw, adw2

| | | | | | | | | | | | |
|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| CAC | ATC | TCG | TCA | ATC | TCC | GCG | AGG | ACT | GGG | GAC | CCT |
| His | Ile | Ser | Ser | Ile | Ser | Ala | Arg | Thr | Gly | Asp | Pro |
| -3.2 | 4.5 | -0.8 | -0.8 | 4.5 | -0.8 | 1.8 | -4.5 | -0.7 | -0.4 | -3.5 | -1.6 |



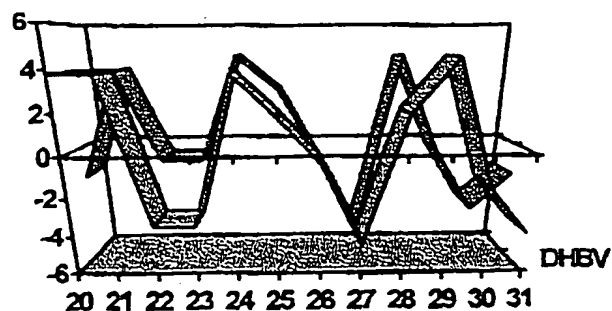
Amphiphilia of PreS2-TLM in various HBV subtypes

Representation of the distribution of the hydrophilic and hydrophobic amino acids in PreS2-TLM in subtype ayw (1) (blue) and the other HBV subtypes ayw (2) (green), adr (1), adr (2) ayr (red) as well as adw and adw2 (gray). The hydropathy values of the amino acid side chains (according to Kyte & Doolittle, 1982) are blotted on the y-axis, positive values correspond to hydrophobic amino acid side chains and negative values correspond to hydrophilic ones. The 12 amino acids of PreS2-TLM and the corresponding sequences of six other subtypes are plotted on the x-axis, the N-terminal proline being located at position 1.

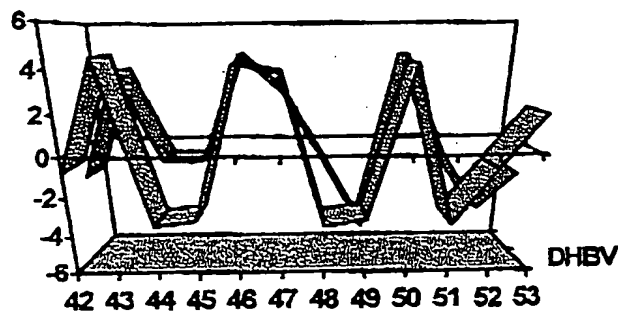
Fig. 3

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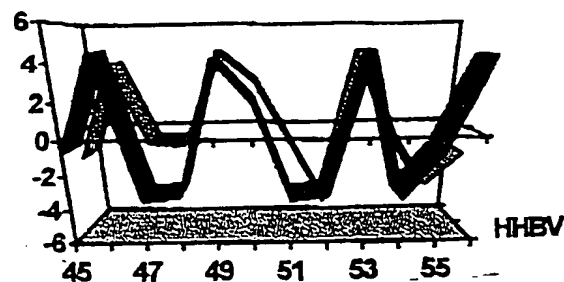
| n | DHBV | PreS2-TLM | DHBV | PreS2-TLM |
|----|---------|-----------|------|-----------|
| 20 | L (Leu) | P (Pro) | 3,8 | -1,6 |
| 21 | L (Leu) | L (Leu) | 3,8 | 3,8 |
| 22 | N (Asn) | S (Ser) | -3,5 | -0,8 |
| 23 | Q (Gln) | S (Ser) | -3,5 | -0,8 |
| 24 | L (Leu) | I (Ile) | 3,8 | 4,5 |
| 25 | A (Ala) | F (Phe) | 1,8 | 2,8 |
| 26 | G (Gly) | S (Ser) | -0,4 | -0,8 |
| 27 | R (Arg) | R (Arg) | -4,5 | -4,5 |
| 28 | M (Met) | I (Ile) | 1,9 | 4,5 |
| 29 | I (Ile) | G (Gly) | 4,5 | -0,4 |
| 30 | P (Pro) | D (Asp) | -1,6 | -3,5 |
| 31 | K (Lys) | P (Pro) | -3,9 | -1,6 |



| n | DHBV | PreS2-TLM | DHBV | PreS2-TLM |
|----|---------|-----------|------|-----------|
| 42 | T (Thr) | P (Pro) | -0,7 | -1,6 |
| 43 | I (Ile) | L (Leu) | 4,5 | 3,8 |
| 44 | D (Asp) | S (Ser) | -3,5 | -0,8 |
| 45 | H (His) | S (Ser) | -3,2 | -0,8 |
| 46 | V (Val) | I (Ile) | 4,2 | 4,5 |
| 47 | L (Leu) | F (Phe) | 3,8 | 2,8 |
| 48 | D (Asp) | S (Ser) | -3,5 | -0,8 |
| 49 | H (His) | R (Arg) | -3,2 | -4,5 |
| 50 | V (Val) | I (Ile) | 4,2 | 4,5 |
| 51 | Q (Gln) | G (Gly) | -3,5 | -0,4 |
| 52 | T (Thr) | D (Asp) | -0,7 | -3,5 |
| 53 | M (Met) | P (Pro) | 1,9 | -1,6 |



| n | HHBV | PreS2-TLM | HHBV | PreS2-TLM |
|----|---------|-----------|------|-----------|
| 45 | T (Thr) | P (Pro) | -0,7 | -1,6 |
| 46 | I (Ile) | L (Leu) | 4,5 | 3,8 |
| 47 | Q (Gln) | S (Ser) | -3,5 | -0,8 |
| 48 | H (His) | S (Ser) | -3,2 | -0,8 |
| 49 | V (Val) | I (Ile) | 4,2 | 4,5 |
| 50 | M (Met) | F (Phe) | 1,9 | 2,8 |
| 51 | D (Asp) | S (Ser) | -3,5 | -0,8 |
| 52 | H (His) | R (Arg) | -3,2 | -4,5 |
| 53 | I (Ile) | I (Ile) | 4,5 | 4,5 |
| 54 | D (Asp) | G (Gly) | -3,5 | -0,4 |
| 55 | S (Ser) | D (Asp) | -0,8 | -3,5 |
| 56 | V (Val) | P (Pro) | 4,2 | -1,6 |

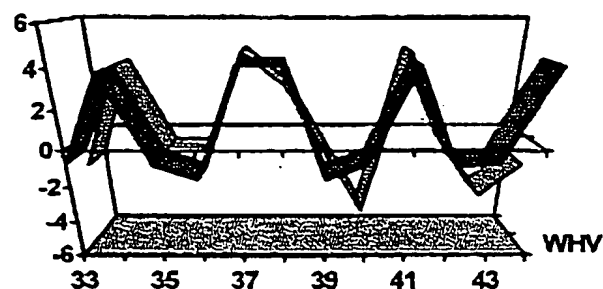


Amphiphilic motives in the PreS region of various avian hepadnaviruses

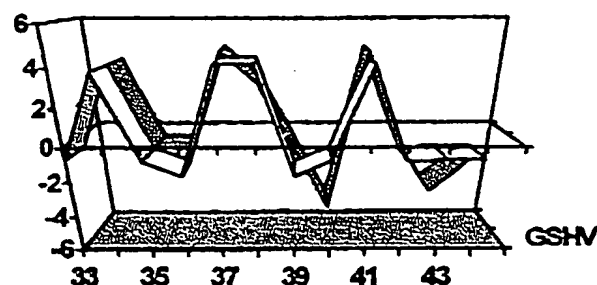
Comparison of the hydropathy profiles of PreS2-TLM (green) with segments of the PreS region of DHBV3 (red) and HHBV (blue). The positions of the amino acids in DHBV3 and HHBV are indicated in the tables (n) together with the amino acid sequence of the corresponding segment and the PreS2-TLM. The hydropathy values are also indicated (according to Kyte & Doolittle, 1982). Motives having a distribution of hydrophobic and hydrophilic amino acids similar to that in PreS2-TLM are found between amino acids 20 to 31 and 42 to 53 of DHBV3 or 45 to 56 of HHBV.

Fig. 4

| n | WHV | PreS2-TLM | WHV | PreS2-TLM |
|----|---------|-----------|------|-----------|
| 33 | T (Thr) | P (Pro) | -0,7 | -1,6 |
| 34 | L (Leu) | L (Leu) | 3,8 | 3,8 |
| 35 | S (Ser) | S (Ser) | -0,8 | -0,8 |
| 36 | P (Pro) | S (Ser) | -1,6 | -0,8 |
| 37 | V (Val) | I (Ile) | 4,2 | 4,5 |
| 38 | V (Val) | F (Phe) | 4,2 | 2,8 |
| 39 | P (Pro) | S (Ser) | -1,6 | -0,8 |
| 40 | T (Thr) | R (Arg) | -0,7 | -4,5 |
| 41 | V (Val) | I (Ile) | 4,2 | 4,5 |
| 42 | S (Ser) | G (Gly) | -0,8 | -0,4 |
| 43 | T (Thr) | D (Asp) | -0,7 | -3,5 |
| 44 | I (Ile) | P (Pro) | 4,2 | -1,6 |



| n | GSHV | PreS2-TLM | GSHV | PreS2-TLM |
|----|---------|-----------|------|-----------|
| 33 | T (Thr) | P (Pro) | -0,7 | -1,6 |
| 34 | L (Leu) | L (Leu) | 3,8 | 3,8 |
| 35 | S (Ser) | S (Ser) | -0,8 | -0,8 |
| 36 | P (Pro) | S (Ser) | -1,6 | -0,8 |
| 37 | V (Val) | I (Ile) | 4,2 | 4,5 |
| 38 | V (Val) | F (Phe) | 4,2 | 2,8 |
| 39 | P (Pro) | S (Ser) | -1,6 | -0,8 |
| 40 | T (Thr) | R (Arg) | -0,7 | -4,5 |
| 41 | V (Val) | I (Ile) | 4,2 | 4,5 |
| 42 | S (Ser) | G (Gly) | -0,8 | -0,4 |
| 43 | T (Thr) | D (Asp) | -0,7 | -3,5 |
| 44 | T (Thr) | P (Pro) | -0,7 | -1,6 |

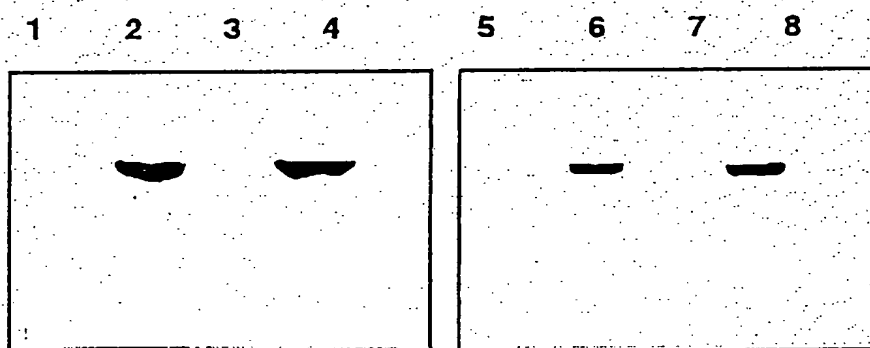


Amphiphilic motives in the PreS2 region of various hepadnaviruses of rodents

A comparison of the hydropathy profiles of PreS2-TLM (green) with segments of the PreS region of WHV (turquoise) and GSHV (yellow). The positions of the amino acids in WHV and GSHV are indicated in the tables (n) together with the amino acid sequence of the corresponding segment and the PreS2-TLM. The hydropathy values are also indicated (according to Kyte & Doolittle, 1982). Motives having a distribution of hydrophobic and hydrophilic amino acids similar to that in PreS2-TLM are found between amino acids 33 to 44 of PreS2 from WHV and GSHV. The motive is conserved between both hepadnaviruses.

Fig. 5

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DHBV42-53-EGFP is a cell-permeable protein

Immunoblot of cytosolic lysates of 293 cells after 10 minutes (1, 2, 5, 6) or 20 minutes of incubation (3, 4, 7, 8) with 1 mM EGFP (1, 3, 5, 7) or 1 mM DHBV 42-53-EGFP (2, 4, 6, 8). For carrying out the immunoblot, an antibody directed against the N-terminal hexa-His-Tag of the recombinant proteins (1-4) or directed against EGFP was used (5-8).

Fig. 6